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EFFECT OF CYSTEINYL LEUKOTRIENE BLOCKADE ON THE DEVELOPMENT OF ACUTE MOUNTAIN SICKNESS

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Acute Mountain Sickness (AMS) may be a manifestation of hypoxia-induced cerebral edema resulting, in part, from increased capillary permeability. Leukotrienes (LTB4, LTC4) may be involved in the pathogenesis of AMS, as these compounds are known to increase endothelial permeability. To test the hypothesis that cysteinyl leukotrienes (LTC4) are involved in the development of AMS, we orally administered a cysteinyl leukotriene receptor blocker (montelukast) prior to and during exposure to high altitude (4300 m) in a hypobaric chamber. We assessed whether blocking cysteinyl leukotriene receptors decreases the prevalence and/or severity of AMS and associated physiological and cognitive responses. Eleven lowlanders (9 men, 2 women) were exposed twice, 2 weeks apart to 4300 m for 24 hours. Each subject received 10 mg montelukast 25 and 1 h prior to ascent. AMS symptoms were assessed using the Environmental Symptoms Questionnaire (ESQ) and the Lake Louise AMS Scoring System (LL) after 11 and 22 h altitude exposure. Urinary LTE4 (uLTE4) was assayed as an integrated measure of cysteinyl leukotriene production. After 11 h exposure, AMS prevalence and symptom severity by LL was lower ($p<0.05$) during montelukast administration compared to placebo during the first 12 h, but not different after 22 h exposure. The LL identified more subjects with AMS than the ESQ, but the scores between the two assessments were highly correlated ($r=0.78$ to 0.98). Urinary LTE4 was not significantly elevated after 24 h exposure, nor did uLTE4 levels correlate with AMS severity. However, uLTE4 tended to be higher ($p=0.06$) during montelukast treatment compared to placebo. Compared to placebo, montelukast administration was not associated with any significant differences in resting SaO2, PETO2, PETCO2, 24h urine volume, TBW or changes in cognitive performance at sea level or high altitude. However, cognitive performance did recover after only 12 hours of altitude exposure. While these results suggest a role for the cysteinyl leukotrienes in the development of AMS, they do not rule out the possible involvement of the non-cysteinyl leukotriene B4 in the etiology of AMS.

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EFFECT OF CYSTEINYL LEUKOTRIENE BLOCKADE ON THE DEVELOPMENT OF ACUTE MOUNTAIN SICKNESS

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BACKGROUND

Mountain environments are likely areas of military confrontation. Mountain ranges typically form the borders of nations, and numerous regions of geopolitical interest to the U.S. such as the Balkans, South America, the Middle East, and Asia contain extensive areas of moderate (>1500 m) to high (>2400 m) altitudes. Rapid force projection to such altitudes presents challenges in sustaining optimal military performance due to the hypoxia associated with altitude exposure and its deleterious affect on mission-related work activities. Acute Mountain Sickness (AMS), caused by hypobaric hypoxia, will negatively impact military operations. Current interventions to prevent AMS include slow or staged ascent and select pharmaceuticals. However, each currently available procedure or medication for prophylaxis or treatment of AMS can constrain or degrade mission effectiveness independent of AMS. Leukotrienes (LTB4, LTC4) are a class of inflammatory mediators that may be involved in the pathogenesis of AMS, as these compounds are known to increase endothelial permeability. Thus, blocking the action of leukotrienes may provide prophylaxis against AMS without producing adverse side effects. Therefore, this study assessed whether blocking cysteinyl leukotriene receptors decreased the prevalence and/or severity of AMS and associated physiological and cognitive responses. The study was funded under U.S. Army Medical Research and Materiel Command Scientific and Technical Objective J with the support of Merck & CO., INC.

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EXECUTIVE SUMMARY

Acute Mountain Sickness (AMS) is a multi-system disorder that is principally characterized by headache, anorexia, nausea, vomiting, insomnia, lassitude, and malaise. The syndrome is common in unacclimatized low altitude residents who rapidly ascend to terrestrial elevations exceeding 2,500 m. The symptoms usually appear within 24 hr of exposure and normally resolve after several days. Acute Mountain Sickness is usually self-limited, but may progress into high altitude cerebral edema (HACE) or high altitude pulmonary edema (HAPE), both of which are life-threatening. Acute Mountain Sickness (AMS) may be a manifestation of hypoxia-induced cerebral edema resulting, in part, from increased capillary permeability. Leukotrienes (LTB4, LTC4) may be involved in the pathogenesis of AMS, as these compounds are known to increase endothelial permeability.

To test the hypothesis that cysteinyl leukotrienes (LTC4) are involved in the development of AMS, we orally administered a cysteinyl leukotriene receptor blocker (montelukast) prior to and during exposure to high altitude (4300 m) in a hypobaric chamber. We assessed whether blocking cysteinyl leukotriene receptors decreases the prevalence and/or severity of AMS and associated physiological and cognitive responses. Eleven lowlanders (9 men, 2 women) were exposed twice, 2 weeks apart to 4300 m for 24 hours. Each subject received 10 mg montelukast 25 and 1 hr prior to ascent. AMS symptoms were assessed using the Environmental Symptoms Questionnaire (ESQ) and the Lake Louise AMS Scoring System (LL) after 11 and 22 hr altitude exposure. Urinary LTE4 (uLTE4) was assayed as an integrated measure of cysteinyl leukotriene production.

After 11 hr exposure, AMS prevalence and symptom severity by LL was lower ($p<0.05$) during montelukast administration compared to placebo during the first 12 h, but not different after 22 hr exposure. The LL identified more subjects with AMS than the ESQ, but the scores between the two assessments were highly correlated ($r=0.78$ to 0.98). Urinary LTE4 was not significantly elevated after 24 hr exposure, nor did uLTE4 levels correlate with AMS severity. However, uLTE4 tended to be higher ($p=0.06$) during montelukast treatment compared to placebo. Compared to placebo, montelukast administration was not associated with any significant differences in resting SaO_2, $PETO_2$, $PETCO_2$, 24-hr urine volume, TBW or changes in cognitive performance at sea level or high altitude. However, cognitive performance did recover after only 12 hours of altitude exposure. While these results suggest a role for the cysteinyl leukotrienes in the development of AMS, they do not rule out the possible involvement of the non-cysteinyl leukotriene B4 in the etiology of AMS.

INTRODUCTION

Acute Mountain Sickness (AMS) is a syndrome that is characterized by headache, anorexia, nausea, vomiting, insomnia, lassitude, and malaise. The syndrome has great individual variation in susceptibility; however, the hypoxia-induced symptoms are most common in unacclimatized low altitude residents who rapidly ascend to terrestrial elevations exceeding 2,500 m (26). In addition, the development of AMS appears to be promoted by engaging in physical activities at high altitude (8). The symptoms of AMS commonly appear within 4 to 24 hr of exposure, and usually resolve after several days as acclimatization to hypoxia is achieved. Acute Mountain Sickness is usually self-limited, but may progress into high altitude cerebral edema (HACE) or high altitude pulmonary edema (HAPE), both of which are potentially life-threatening.

Although there has been much speculation about the cause of AMS symptoms, little definitive information exists. The most widely accepted hypothesis is that the symptoms are a manifestation of hypoxia-induced, subclinical cerebral edema that causes swelling of the brain (12,19,38,39). This hypothesis is based primarily upon the observations that: 1) clinically apparent HACE is usually preceded by AMS symptoms (11,38), 2) AMS is often associated with other altitude-induced edematous conditions (HAPE and/or peripheral edema) (11,38,39), and 3) AMS symptoms resolve with a diuresis, which is interpreted as a sign of resolving edema (11,38). Several studies (20,23,25) using non-invasive imaging have reported changes in brain matter density or volume consistent with cerebral edema. We recently reported (25) that in sea level residents exposed to 4,500 m for 32 h, MRI results revealed a consistent and reproducible increase in brain volume, primarily of the gray matter, consistent with development of diffuse cerebral edema. However, there was no apparent relationship between the AMS prevalence or severity and the magnitude of the brain volume changes. Nevertheless, in total, these studies point towards disruption of cerebral tissue fluid distribution as the likely cause of AMS.

Increased cerebral extracellular fluid volume may result from vasogenic edema caused by increasing capillary pressure and/or capillary permeability. One study has reported a significant correlation between the increase in cerebral blood flow and presence of AMS (4) while others have not (13,16,29). Nonetheless, increased cerebral blood flow is a relatively common occurrence at high altitude (3,13-16,29). A number of observations suggest that inflammation may also be an important factor in the pathogenesis of AMS. In support of this concept is the observation that the synthetic corticosteroid dexamethasone is effective in the prophylaxis and treatment of AMS (6,10,27,28). Dexamethasone blocks the formation of free arachidonic acid through an indirect effect on phospholipase A2, leading in turn to decreased levels of both prostaglandins and leukotrienes. Naproxen, a known inhibitor of cyclooxygenase (prostaglandin synthesis) has no beneficial effect on AMS (24). This implies that products of the lipoxygenase pathway (primarily leukotrienes) are responsible for causing the symptoms of AMS.

Patients with diseases that involve inflammation, such as asthma, have elevated levels of leukotriene E4 (LTE4) in their urine (40,41). LTE4 is the metabolite of the more

potent leukotrienes C4 and D4 (5). An elevated urinary LTE4 level may in fact be a marker of generalized inflammation. That leukotrienes may be involved in the pathogenesis of AMS and HAPE is biologically plausible, as these compounds are known to increase endothelial permeability (5,7,22), an important contributor to AMS and HAPE. Several studies have reported increased presence of leukotrienes concomitant with the development of AMS and HAPE.

Studies examining the bronchoalveolar lavage of victims of HAPE have shown fluid that is rich in neutrophils and proteins (37). Elevated levels of leukotriene B4 (LTB4) and complement have also been found (36). In addition, many authors have described persistent pulmonary hypertension and infiltrates despite resolution of hypoxemia. This observation implies that an ongoing inflammatory process in the lungs is still operative. Indeed, we found (18) that urinary LTE4 levels were elevated in 38 HAPE subjects compared to 10 control subjects present at the same altitude in Summit County, CO.

Richalet et al. (31) reported that serum levels of LTB4, LTC4 (which is metabolized to LTE4, then excreted in the urine), and prostaglandin E2 increased in humans who rapidly ascended and resided at 4,350 m, generally peaking within 24 to 48 hr after arrival. Their data also suggested a relationship between the levels of these mediators and AMS symptoms. Subsequently, we found (32) that urinary LTE4 levels increased after ~12 hr residence at 4,300 m. Furthermore, the data suggested a correlation between urinary LTE4 levels and AMS symptoms. However, the strength of that correlation was limited due to the relatively low occurrence of AMS in that study.

OBJECTIVE

This study tested the hypothesis that the cysteinyl leukotrienes (LTC4, LTD4) are involved in mediating the development of AMS. To test this hypothesis, we administered montelukast, a specific cysteinyl leukotriene receptor blocker, in a randomized, double-blinded, placebo-controlled crossover trial at 4300 m altitude. The specific objectives of this study were: 1) to assess the absence or presence and severity of signs and symptoms constituting the symptom complex described as AMS in sea level residents during 24 hr exposure to 4300 m with and without pharmacologic cysteinyl leukotriene receptor blockade, 2) measure specific ventilatory, cardiovascular, body fluid and other physiologic parameters indicative of the early acclimatization process in volunteers during 24 hr exposure to 4300 m with and without pharmacologic cysteinyl leukotriene receptor blockade, 3) measure markers of inflammation and hypoxic stress, and 4) measure specific aspects of cognitive performance with and without pharmacologic cysteinyl leukotriene receptor blockade.

METHODS

SUBJECTS

Twelve volunteers enrolled in this study. All gave written and verbal acknowledgment of their informed consent and each was made aware of their right to withdraw without prejudice at any time. Each was a lifelong low altitude resident and had no exposure to altitudes greater than 1000 m for at least 6 months immediately preceding the study. All volunteers received medical examinations, and none were found to have any condition that would warrant exclusion from the study. All were U.S. Army personnel that participated in regular physical training and were of average fitness. Eleven volunteers (9 male, 2 female) completed all elements of the protocol. The 11 volunteers had a mean (±SD) age, height and body weight of 24 ± 4 yrs, 175 ± 8 cm, and 81 ± 13 kg.

PROTOCOL

The study design was a randomized, double-blinded, placebo-controlled crossover trial. The test volunteers were exposed sequentially to sea level and high altitude for testing purposes in the USARIEM hypobaric chamber facility in Natick, MA on two occasions (Test Phases), once while on the leukotriene antagonist and once on the placebo. The sea-level exposure was 24 hr in length and immediately preceded the altitude exposure. Sea-level exposure was at ambient barometric pressure (~760 mmHg) in the hypobaric chamber. Testing at sea-level pressure functioned as the control condition for subsequent altitude (hypobaric) testing. Following completion of the sea-level testing, the chamber containing the volunteer subjects was decompressed at a rate of 45 mmHg•min^{-1} to a pressure of 446 mmHg, which is approximately equivalent to a terrestrial altitude of 4300 m (14,110 ft). The subjects remained at that altitude continuously for 24 hr until the completion of testing. Following completion of the altitude testing, the chamber was recompressed to ambient barometric pressure.

The temperature and relative humidity in the chamber was maintained at 21 ± 2°C and 45 ± 5% respectively for all testing. Volunteers were allowed to participate in sedentary activities (reading, TV viewing) when not involved in actual testing during each Test Phase. For logistical purposes, volunteers were exposed to sea-level and altitude conditions in groups of 5-6 individuals.

For each test volunteer the study schedule required about 6 weeks, and included a Preliminary Phase, the two Test Phases and intervening recovery periods. During the Preliminary Phase (~3 days), volunteers received required hypobaric chamber orientation and safety training were familiarized with the test procedures and had a short (~6 h) exposure in the hypobaric chamber. The purpose of this short high altitude exposure was to familiarize the subjects with the perceptual cues associated with the chamber environment and perceived symptoms related to the hypobaric hypoxia and exercise stresses. We expected that this familiarization would reduce the "novelty factor" influence

on the reporting of subjective symptoms during the subsequent two Test Phase high altitude exposures. Following the Preliminary Phase, the volunteers had at least a 7-day recovery period to avoid any carryover effects from the first altitude exposure into the first Test Phase. The two Test Phases were each 4 days in length and were separated by a 12-14 day recovery period to avoid carryover effects.

Pharmacologic blockade of cysteinyl leukotriene receptors was produced by ingestion of montelukast sodium. Montelukast (trade name Singulair, Merck & CO., INC.) is a cysteinyl leukotriene antagonist that is approved by the U.S. Food and Drug Administration for clinical use for the prophylaxis and chronic treatment of asthma (1). Montelukast is an orally active compound that binds with high affinity and selectivity to cysteinyl leukotriene receptors. Following oral administration, it is rapidly absorbed and reaches peak plasma concentration in 3 - 4 hours. The mean plasma half-life of montelukast ranges between 2.7 - 5.5 hours in normal healthy adults. Montelukast is more than 99% bound to plasma proteins and has minimal distribution across the blood-brain barrier. Although doses up to 200 mg/day have been tested, no clinical benefit has been observed at doses above 10 mg once daily. The first dose of montelukast (10 mg film-coated tablet) was given at 0800 hr at the beginning of a Test Phase and the second 10 mg dose was given about 24 hr later just prior to decompressing the chamber to simulated altitude. During the placebo trial, an identical appearing tablet containing lactose was ingested on the same schedule during its corresponding Test Phase.

To minimize the effects of diet on gastrointestinal symptoms and loss of appetite associated with AMS, during both Test Phases each volunteer was required to consume a pre-selected diet and to drink a minimum amount of fluid each day. The diet consisted of commercially prepared food items and non-prepared items of known energy and nutritional content. Each volunteer selected his or her choices for each meal from a menu prior to the first Test Phase. They were given identical meals during the second Test Phase.

Since military duties normally require physical work and development of AMS is promoted by physical activity at high altitude, each volunteer performed a circuit of resistive and aerobic exercises for ~2 hours, including rest breaks, during the high altitude exposures. Resistive exercise consisted of bench press and arm curl exercises. Each resistance exercise was performed over 20 minutes in 5 sets. Each set was 8 - 10 repetitions of a 12 repetition max workload. In between each set, the volunteer rested ~3 minutes. The aerobic exercise was a 20-minute submaximal, steady-state exercise bout on a bicycle ergometer at 70% of the subject's predicted heart rate maximum. Heart rate (HR) was measured by three-lead ECG, and SpO_2 was monitored by finger pulse oximetry (Nellcor N-200). During the cycle exercise period, plasma volume and plasma protein concentration were measured by venous blood sampling.

MEASUREMENTS

Procedures and measurements performed during the two Test Phases are listed in Table 1 and described below.

Table 1. Schedule of procedures and measurements during the two Test Phases.

TEST PROCEDURE	TEST DAY 1	2	3	4
montelukast / placebo administration	x	x		
controlled diet	x	x	x	x		
24 hr urine collection	x	x	x	x		
Venous blood sampling		x	x	x		x
AMS symptom assessment	x	x	x	x		
Heart rate, SaO2 measurements	x	x	x	x		
TBW by bioelectric impedance		x		x		
Transthoracic impedance		x		x		
Resting ventilation		x		x		
Pulmonary function		x		x		
Cognitive performance tests	x	x	x	x		
Exercise bout			x			

Sea Level
4,300 m

The incidence and severity of AMS was determined from information gathered using the Environmental Symptoms Questionnaire (ESQ), and the Lake Louise AMS Scoring System (LL). The ESQ is a self-reported, 68-question inventory used to document symptoms induced by altitude and other stressful environments (34). A weighted average of scores from "cerebral" symptoms (headache, lightheaded, dizzy, etc.) designated AMS-C and from "respiratory" symptoms (short-of-breath, hurts-to-breathe, etc.) designated AMS-R were calculated post hoc. AMS-C scores greater than 0.7 and AMS-R scores greater than 0.6 are defined as indicating the presence of AMS. The LL consists of a five question self-reported assessment of AMS symptoms and a three question objective assessment of clinical signs (33). It appears to successfully detect AMS and correlates with ESQ scores (35). The total LL score consisting of the sum of the self-assessment question score and the clinical-assessment score was calculated for each subject post hoc. The ESQ and LL were administered twice during the Preliminary Phase to familiarize the test volunteers with the procedures. The ESQ was administered at 1, 2, 4, 10, 12, 20 and 22 hours into each 24-hr long sea level and high altitude exposure. The LL was administered at 12 and 22 hours into each 24-hr long sea level and high altitude exposure. Coincident with the administration of the ESQ, resting arterial oxygen saturation (SaO_2) and HR were obtained by pulse oximetry.

Resting ventilation measurements were made in each Test Phase once at sea level and again at high altitude in the morning prior to breakfast. All ventilatory tests were performed with the volunteers resting in a seated position. The volunteer breathed through a low-resistance respiratory valve and breathing circuit connected to a computer-controlled, breath-by-breath metabolic measurement system (Vmax229, SensorMedics Corp). Resting ventilation tests measured breath-by-breath: minute ventilation (VE), oxygen uptake (VO_2), carbon dioxide elimination (VCO_2), and partial pressure end-tidal oxygen and carbon dioxide ($PETO_2$ and $PETCO_2$). Simultaneously, arterial oxygen saturation (SaO_2) and pulse rate were measured by finger pulse oximetry (Nellcor N-200). Resting ventilation tests were about 20 min in duration. Resting ventilatory parameters were obtained and mean values calculated from the last 8-10 min of the session. Following the resting ventilatory studies, flow-volume loops were obtained to assess possible altitude-induced changes in Forced Vital Capacity (FVC). The procedure was performed three to five times until reproducibility criteria (9) were achieved.

During the two Test Phases, venous blood samples were obtained after arousal on the mornings of day 2 and day 3, immediately before and after the cycle exercise on day 2 at high altitude, and 24 hr after completion of the altitude exposure (day 4). The morning samples of the whole blood were immediately analyzed for hemoglobin (Hb) and hematocrit (Hct). The remaining blood was processed and stored for later analysis of eicosanoids. The plasma protein samples were obtained from an indwelling catheter after the subject rested upright on the cycle ergometer for 20 min pre-exercise and during the last minute of exercise. Body fluid status was assessed by measuring 24-hour urinary volume, daily semi-nude (i.e., T-shirt and shorts or swim suit) body weight, bio-impedance measurements for total body water and urinary and plasma osmolality (U_{osm}, V_{osm}). Twenty-four hour urine volume was calculated from measurement of separate 12-hr urine collections. Aliquots of the 12-hr urine collections will be taken for analysis of creatinine (U_{creat}) and U_{osm}. Urinary LTE4 concentrations were determined by reverse-phase HPLC and ELISA in aliquots of the second 12-hr urine collections at sea level and high altitude.

Bioelectric-impedance was used to estimate changes in total body water of the volunteer subjects. The volunteers were tested after waking in the morning of days 2 and 3. The measurement was made after 10 minutes of supine rest. An electric current (800 μA maximum at 50 kHz) was passed through their body from electrodes on the hand to similar electrodes on the ankle (RJL Systems, BIA-101). Resistivity was corrected for changes in Hct. Immediately following the bioelectric-impedance measurement, an estimate of thoracic fluid volume was obtained by transthoracic impedance (TTI). The TTI data was collected using four resting ECG electrodes: two stimulating electrodes (forehead and ankle), and 2 signal pick-up electrodes 3 cm to the right of the first thoracic vertebrae and 30 cm directly below (Cardiograph Impedance IFM, model 304B).

To determine if cysteinyl leukotriene blockade decreases the adverse changes in cognitive function caused by hypoxia, the volunteers completed The Spaceflight Cognitive Assessment Tool (SCAT), which contains a Running Memory Task, Math Task, Match to Sample Task, and Code Delayed Task (30). Subjects completed this series of tasks using a Panasonic™ Laptop computer; model CF-41, with colored displays while seated at a

table. All volunteers were administered the SCAT simultaneously during each Test Phase. The volunteers were given about 10 administrations of distributed practice during the Preliminary Phase of the study. The SCAT was administered at 0.5, 2, 4, 12 and 23 hours into each 24-hour long sea level and high altitude exposure.

STATISTICAL ANALYSIS

First, two-way (cysteinyl leukotriene receptor blockade and altitude exposure duration) analysis of variance with repeated measures in both factors was used to analyze the data. Data that deviated significantly from normality or failed to meet the qualifying assumptions of analysis of variance were analyzed using appropriate non-parametric techniques (i.e., ANOVA on ranks, or Mann-Whitney rank sum test, etc). Second, the subjects were then divided into two groups depending upon whether they developed or did not develop AMS (*AMS+*, or *AMS-*). High altitude measurements of the *AMS+* and *AMS-* groups within each drug treatment were compared using the Student T-test, or if the data deviated significantly from normality or failed to meet the qualifying assumptions of analysis of variance, the data were analyzed using the Mann-Whitney Rank Sum Test. Potential relationships between measured parameters were tested using the Pearson Product Moment Correlation. Statistical significance was accepted at $p \leq 0.05$. Values are presented as mean ± standard deviation (SD).

RESULTS

During the placebo trial, 7 of the 11 volunteers developed AMS assessed by ESQ-C scores while 9 developed AMS according to the LL AMS score (Table 2). During the montelukast trial, by both the ESQ-C and LL AMS scores, AMS developed in the same 7 volunteers in which it was observed during the placebo trial. The severity of AMS, as measured by LL AMS scores, was significantly less ($p<0.05$) during the first 12 hours of altitude exposure in the montelukast compared to the placebo trials (Figure 1). However, by 22 hours exposure, AMS severity by the LL AMS score was not different between drug treatments and AMS severity assessed by ESQ-C was never significantly different between trials during any measurement period (Figure 1). There was a wide range in interindividual AMS severity during both trials. This is illustrated in Figure 2, which plots the cumulative AMS symptom scores for each individual in both trials. These plots clearly show that volunteers with the highest AMS symptom severity in the placebo trial experienced the greatest decrease in symptom severity in the montelukast trial after both 12 and 22 hours of altitude exposure. None of the physiological parameters measured at sea level or during the altitude exposure were significantly different between the placebo and montelukast trials (Table 2).

Table 2. AMS Scores and physiological parameters at high altitude in the placebo and montelukast trials.

Variable	Placebo Trial	Montelukast Trial
AMS+ ESQ-C (#Ss)	7	7
AMS+ LL AMS (#Ss)	9	7
SQ-C Score	0.69±1.00	0.52±0.55
LL AMS Score	2.6±3.7	1.9±3.2
SL $uLTE_4$ ($pg \cdot mg^{-1}$)	80.5±36.5	79.4±38.5
HA $uLTE_4$ ($pg \cdot mg^{-1}$)	90.2±39.7	101.8±48.0
SaO_2 (%)	82±5	82±3
$PETO_2$ (mmHg)	47.6±4.3	48.0±4.2
$PETCO_2$ (mmHg)	31.7±4.0	32.1±2.7
FEV1 (l)	3.96±0.64	3.98±0.75
Hb ($g \cdot dl^{-1}$)	15.2±1.3	15.2±1.07
Hct (%)	46.7±3.4	46.9±3.0
V_{osm} (mOsmol)	280±4	281±3
U_{osm} (mOsmol)	575±289	438±228
24-hr Uvol (l)	2.6±1.6	3.0±1.9
ΔBW (kg)	0.06±0.59	0.07±0.95
TBW (l)	39.1±6.1	39.6±6.1
TTI (ΔZo)	0.28±1.3	-0.62±1.4

To determine if physiological differences existed between volunteers who were and were not susceptible to AMS, within each trial, the volunteers were partitioned into those who developed AMS (AMS+) and those who did not (AMS-). These data are presented in Table 3. In the placebo trial, resting SaO_2 was significantly lower in the AMS+ group compared to the AMS- group. This SaO_2 difference was not present in the montelukast trial, nor were any other significant physiological differences observed between the AMS+ and AMS- volunteers.

Table 3. AMS Scores and physiological parameters at high altitude segregated by AMS susceptibility in the placebo and montelukast trials.

	Placebo		Montelukast	
Variable	AMS+	AMS-	AMS+	AMS-
ESQ-C (#Ss)	7	4	7	4
LL AMS (#Ss)	9	2	7	4
ESQ-C Score	1.05±0.75*	0.07±0.02	0.76±0.39*	0.10±0.10
LL AMS Score	7.1±3.4*	1.5±0.7	5.6±3.2*	1.2±1.0
SL $uLTE_4$ ($pg \cdot mg^{-1}$)	86.0±42.7	71.0±24.3	86.4±40.9	67.0±35.8
HA $uLTE_4$ ($pg \cdot mg^{-1}$)	96.0±44.6	80.0±32.3	104.4±48.2	97.0±54.7
SaO_2 (%)	80±4*	85±3	82±3	83±5
$PETO_2$ (mmHg)	46.6±4.9	49.4±2.7	48.1±5.2	47.8±2.3
$PETCO_2$ (mmHg)	31.4±4.3	32.4±3.9	31.6±2.5	33.1±3.0
FEV1 (l)	4.43±0.63	3.69±0.52	4.22±0.79	3.83±0.74
Hb ($g \cdot dl^{-1}$)	15.6±0.9	14.7±1.8	15.5±0.77	14.6±1.4
Hct (%)	47.3±3.0	45.9±4.3	46.8±1.9	47.1±4.7
V_{osm} (mOsmol)	280±2	280±6	281±3	280±3
U_{osm} (mOsmol)	564±296	595±321	484±280	359±51
24-hr Uvol (l)	2.4±1.4	2.8±1.9	3.0±2.1	2.9±1.9
ΔBW (kg)	-0.04±0.22	0.25±0.99	-0.20±0.70	0.55±1.24
TBW (l)	38.4±5.4	40.4±7.8	40.3±5.3	38.2±7.9
TTI (ΔZo)	0.31±1.6	0.22±0.50	-0.79±1.6	-0.34±1.3

*$P<0.05$ AMS+ vs. AMS-

Interindividual levels of uLTE4 were widely dispersed at sea level and high altitude during both trials. As shown in Figure 3, there was a strong correlation ($r = 0.91$, $p<0.001$) between individual sea level uLTE4 and high altitude uLTE4 levels. However, uLTE4 levels following 12 – 24 hours at high altitude were not significantly different from sea level values (Tables 1, 2) in either trial. AMS symptom severity scores by either ESQ-C or LL AMS were not correlated with either the pre-ascent sea level or high altitude uLTE4 levels in either trial. This lack of relationship between uLTE4 and AMS symptom severity is illustrated in Figure 4.

Cognitive performance impairments were evident on all tasks beginning 0.5 hr after ascent and increased through 4 h; at 12 h, effects did not differ from data collected at 30 m (sea level). These trends were most marked for the Code Substitution and the Running Memory Tasks (Figures 5, 6) and statistically different from sea level control values where maximal impairments were 15-20% below the control values. Impairments on the Mathematics and Matching Tasks (Figures 7, 8) were neither statistically significant from

control values nor distinct as those for the Code Substitution and the Running Memory Tasks. On all four SCAT tasks, 4 hr was the time of maximal effects. Treatment with montelukast did not improve cognitive performance.

Figure 1. AMS severity scores after 12 and 22 hours exposure to 4300m in placebo and montelukast trials. * *P*<0.05.

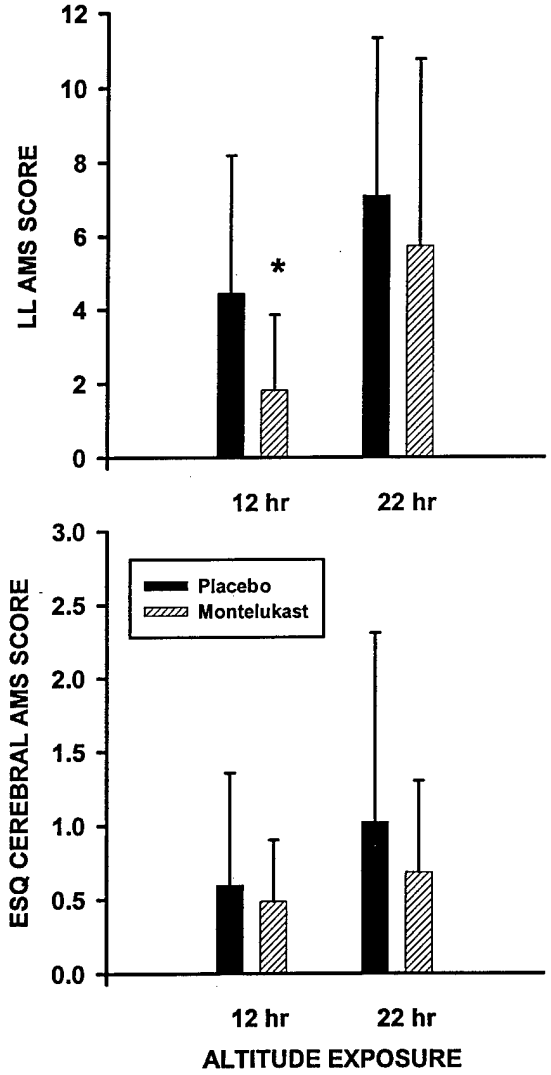


Figure 2. Scatter plots showing relationship between individual AMS severity scores during placebo and montelukast trials. Line of identity plotted for reference.

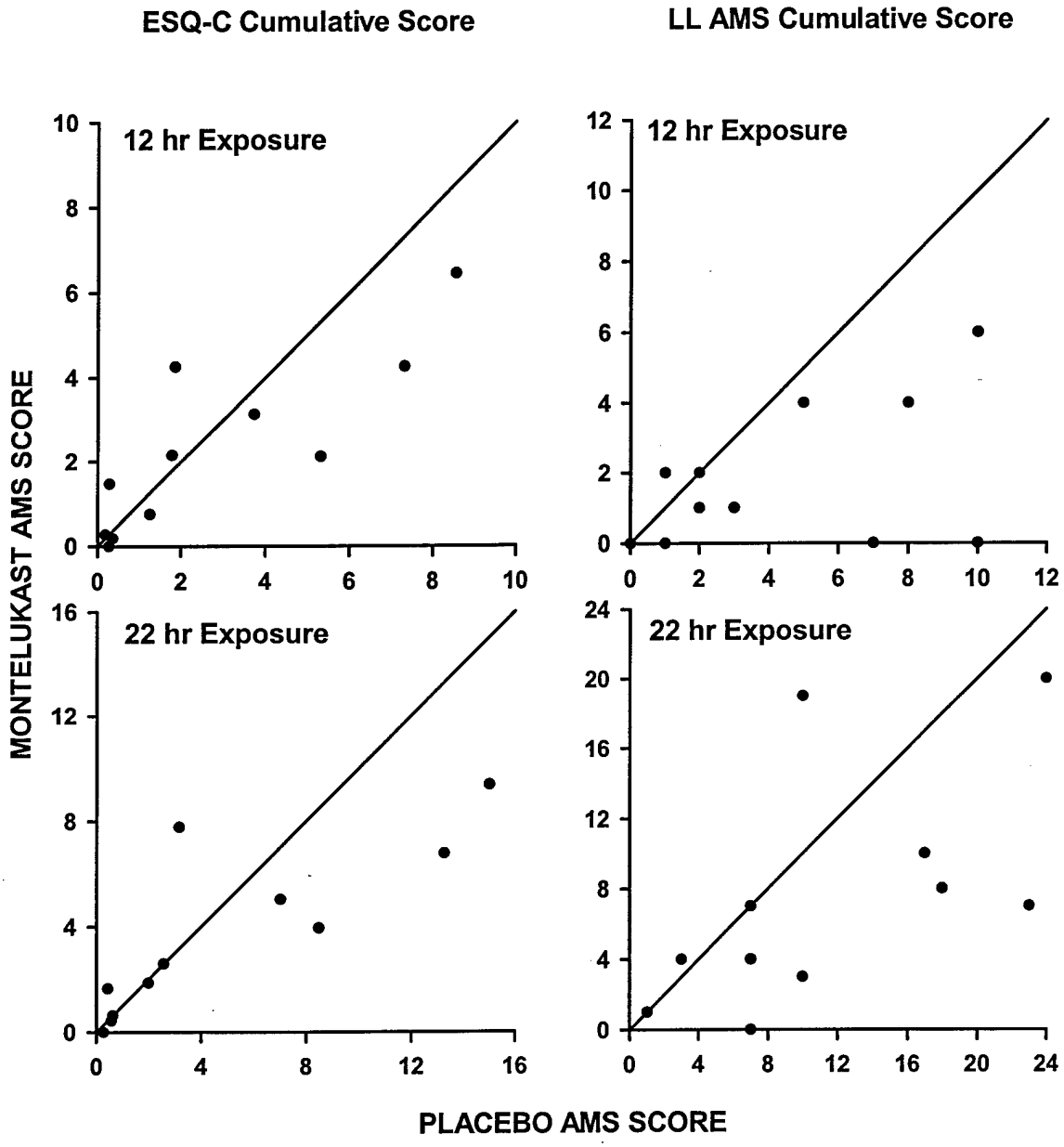


Figure 3. Scatter plot showing relationship between individual uLTE4 levels at sea level and after 24 hours at 4,300 m. Line of identity plotted for reference.

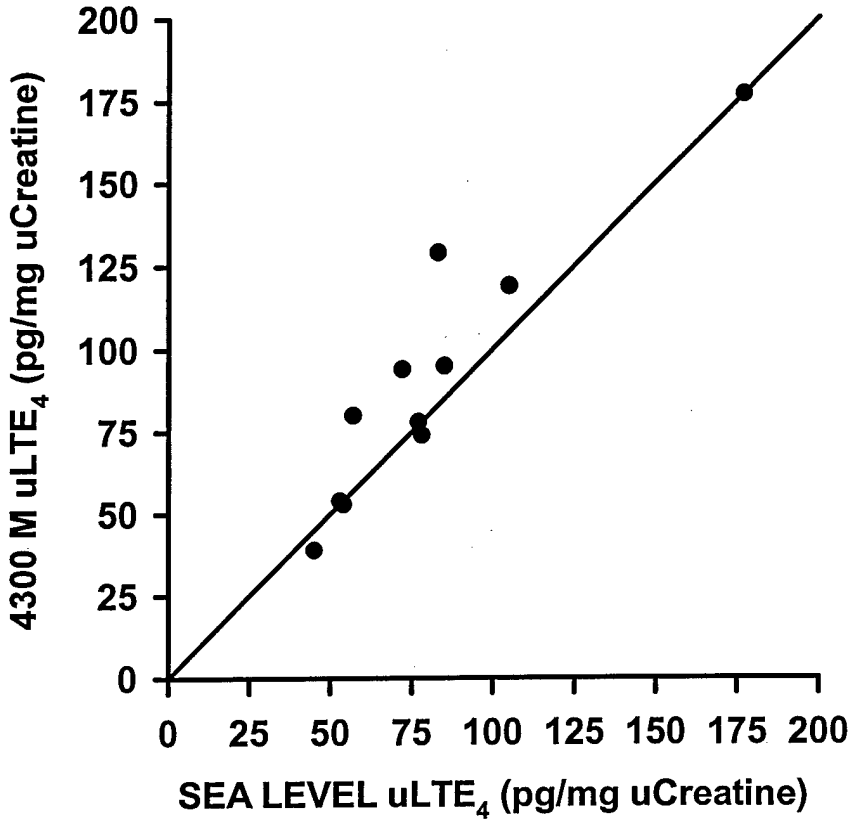


Figure 4. Scatter plot of individual AMS cumulative scores (ESQ-C) plotted as a function of high altitude uLTE4 levels.

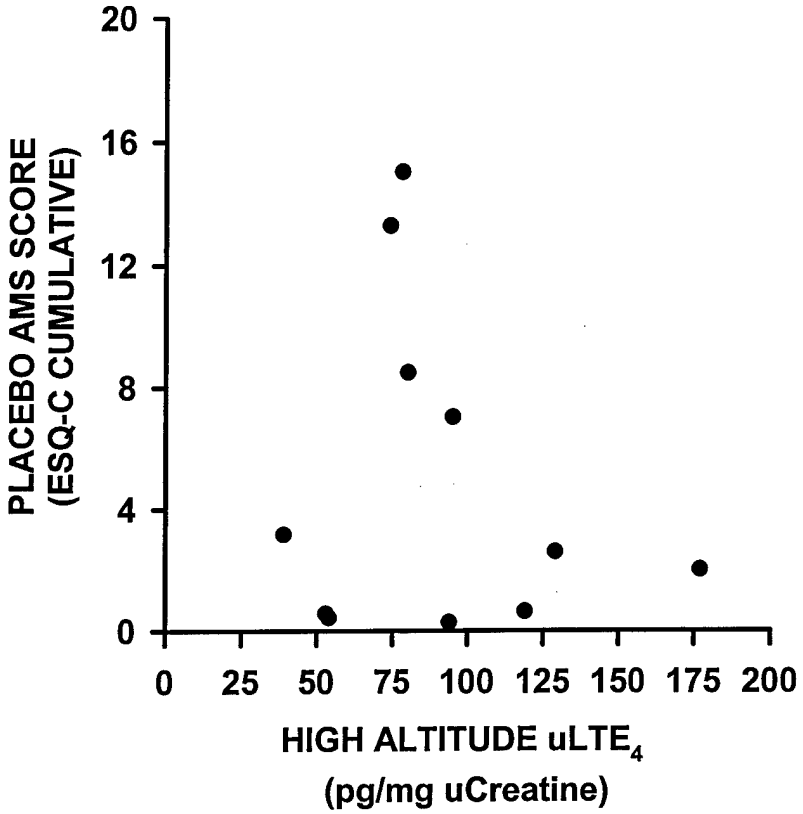
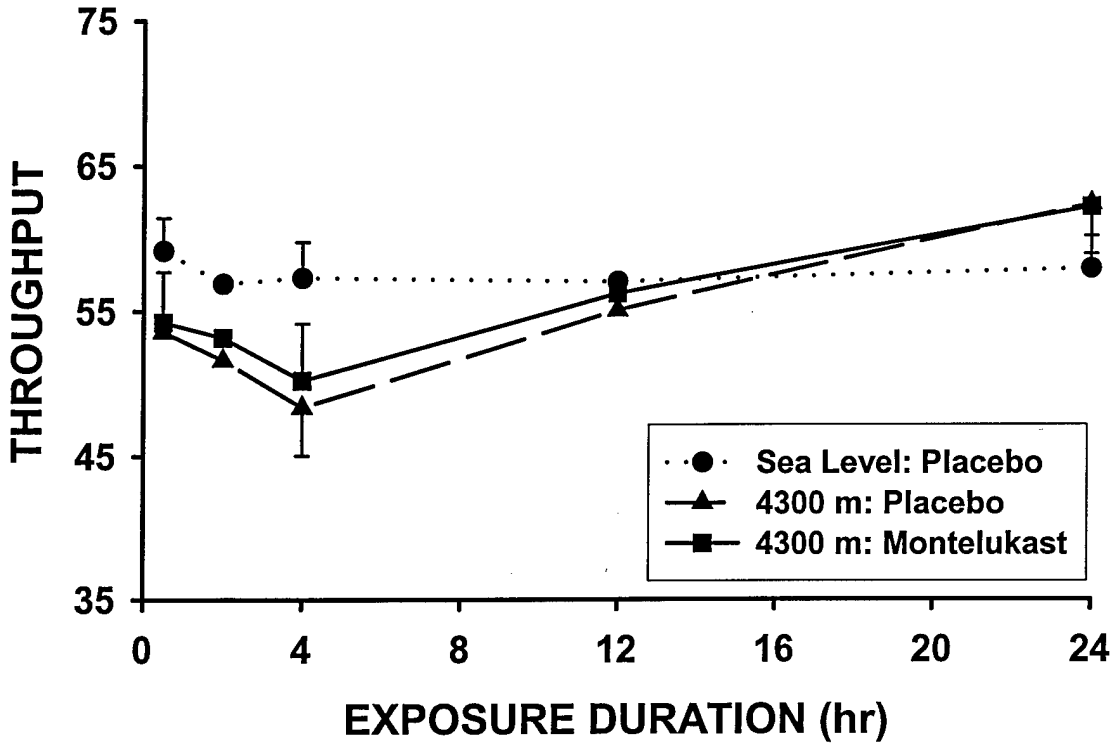
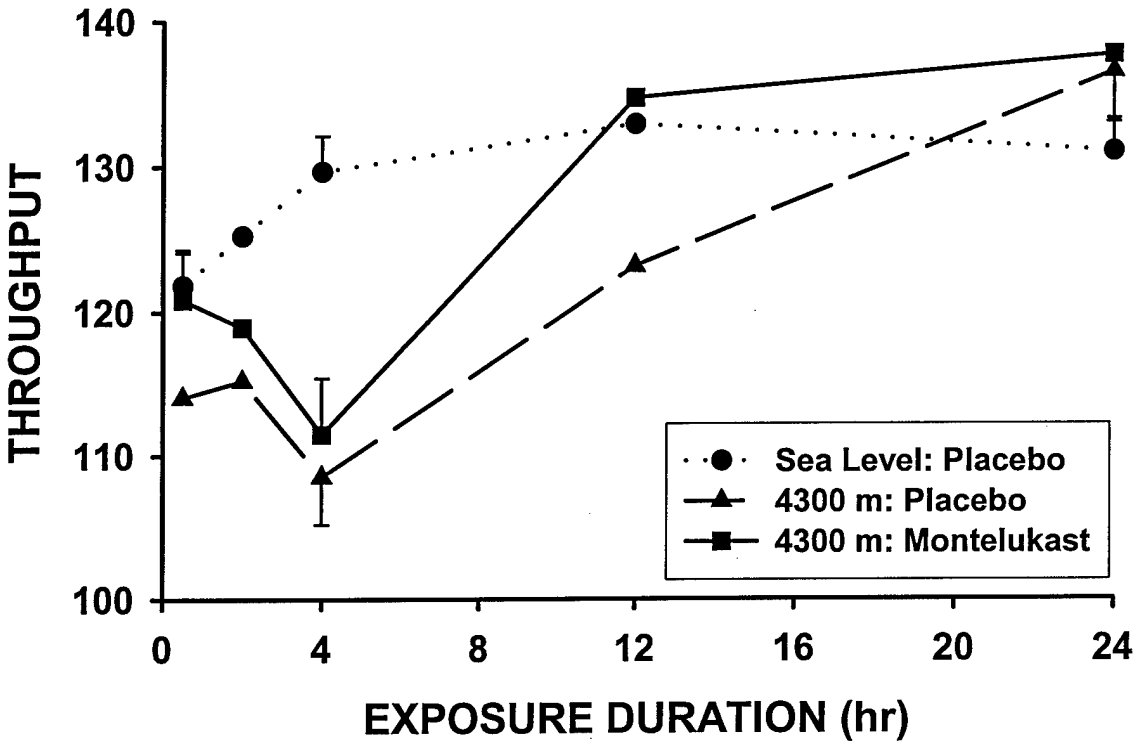


Figure 5. Effect of altitude exposure duration and drug on Code Substitution Task throughput.



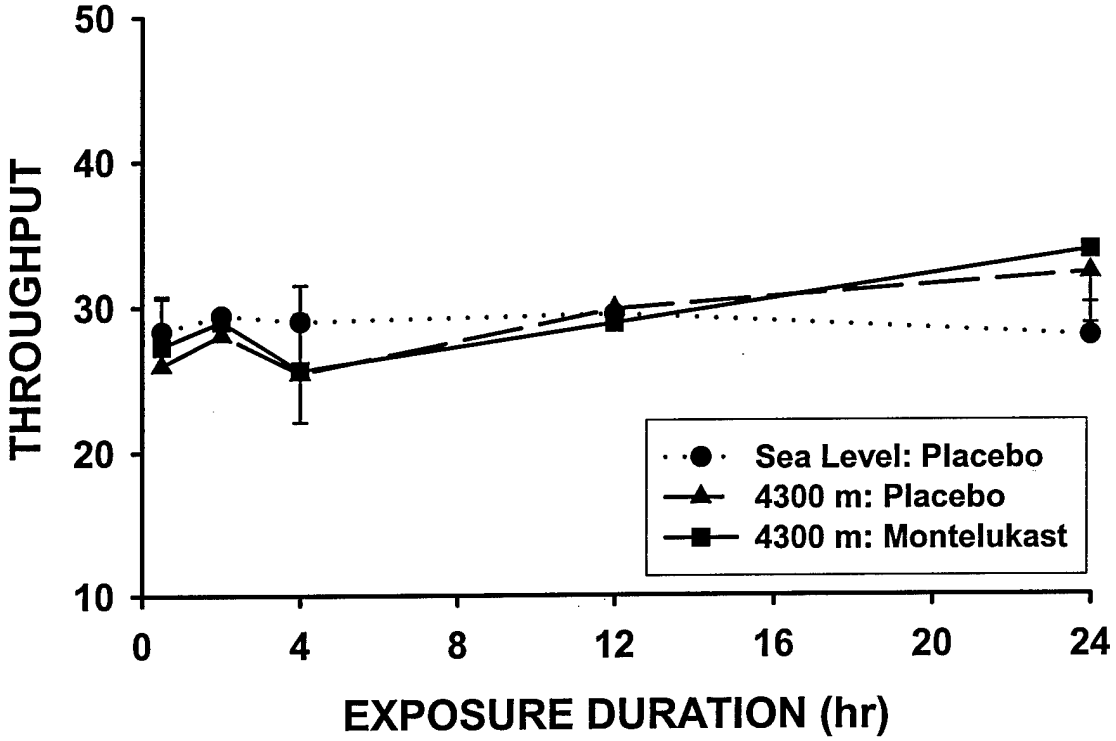
Throughput quantifies the timeliness and accuracy of the task performance, and is expressed in arbitrary units.

Figure 6. Effect of altitude exposure duration and drug on Running Memory Task throughput.



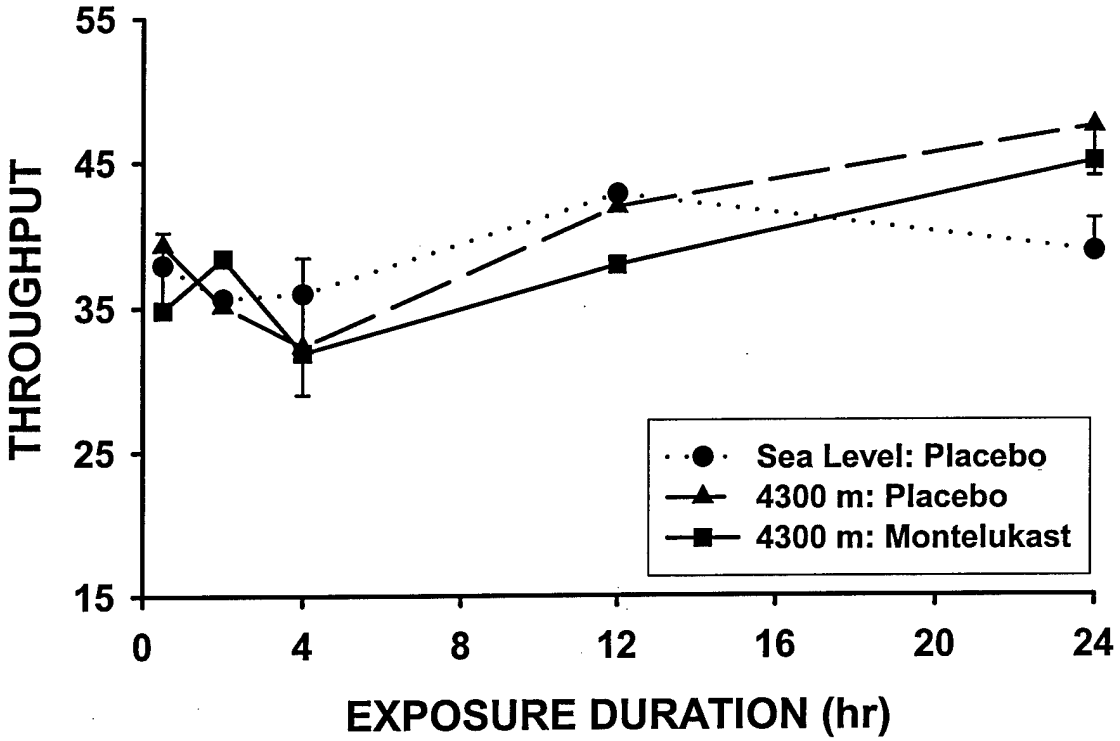
Throughput quantifies the timeliness and accuracy of the task performance, and is expressed in arbitrary units.

Figure 7. Effect of altitude exposure duration and drug on Math Task throughput.



Throughput quantifies the timeliness and accuracy of the task performance, and is expressed arbitrary units.

Figure 8. Effect of altitude exposure duration and drug on Matching Task throughput.



Throughput quantifies the timeliness and accuracy of the task performance, and is expressed in arbitrary units.

DISCUSSION

This study tested the hypothesis that the cysteinyl leukotrienes (LTC4, LTD4) are involved in mediating the development of AMS. To test this hypothesis, we administered montelukast, a specific cysteinyl leukotriene receptor blocker, in a randomized, double-blinded, placebo-controlled crossover trial at 4300 m altitude. Consistent with our hypothesis, AMS prevalence and symptom severity by LL was lower during montelukast administration compared to placebo during the first 12 hr exposure, but not different after 22 hr exposure. On the other hand, uLTE4 was not significantly elevated after 24 hr exposure, nor did uLTE4 levels correlate with AMS severity suggesting that LTC4 or LTD4 were not mediating the development of AMS. Thus, the results suggest a role for the cysteinyl leukotrienes in the development of AMS, but do not rule out the possible involvement of other leukotrienes in the etiology of AMS.

All studies of AMS are beset by two limitations that must be considered in the interpretation of the results. The first is that susceptibility to AMS is not uniform among all volunteers and the second is that the quantifiable assessment of AMS is dependent upon each volunteer's subjective reporting of their symptoms. The ideal test of a pharmacological intervention to prevent or reduce symptoms of AMS would only use a subject population with known, uniform susceptibility to AMS. However, lacking that population, under the ascent conditions used in this study between 60–75% of the volunteers were expected to experience some degree of AMS (17,21), and according to the results apparently did. Thus, only the volunteers who reported experiencing AMS during the placebo trial (7 by ESQ-C, 9 by LL) are useful in assessing whether the pharmacological intervention is effective. However, even among these AMS-susceptible volunteers, the AMS severity varied from very mild to severe. Consequently, the reduction in subject number and large variance in the symptom severity decrease the statistical power to detect potentially clinically important effects of the pharmacological intervention.

Two instruments were used to assess AMS severity, the ESQ-C and LL AMS scores. Both instruments are questionnaires that ask volunteers to assign a categorical rating to symptoms associated with AMS. The LL identified more subjects with AMS than the ESQ, but the scores between the two assessments were highly correlated ($r=0.78$ to 0.98). Thus, the threshold for assigning a diagnosis of AMS appears to be lower using the LL compared to the ESQ-C AMS scores. Consequently, due to the larger number of AMS susceptible subjects per LL AMS scores, a statistically significant reduction in AMS symptom severity was observed in the montelukast trial compared to the placebo trial. The significant reduction in AMS severity was observed after 12 hours altitude exposure (Figure 1). Moreover, it is clear even from the ESQ-C AMS scores that individuals with the highest AMS severity experienced the greatest reduction in AMS severity during the montelukast administration (Figure 2). Thus, the results support our hypothesis that blocking the action of cysteinyl leukotrienes will decrease the prevalence and severity of AMS.

The amelioration of AMS severity by montelukast was absent after 20 hours altitude exposure. Two competing processes may be responsible for this observation. First, AMS severity increases with duration of altitude exposure and is accentuated following sleep

(17,21). Second, given that the volunteers received their last dose of montelukast about 22 hours earlier, the plasma montelukast concentration may have decreased below its therapeutic level. The montelukast dosage used in this study is the FDA recommended dosage for the approved use of this drug in treating asthma (1). Although doses up to 200 mg/day have been tested without significant adverse events, no clinical benefit has been observed at doses above 10 mg once daily. However, it is possible that for prophylaxis of AMS a higher dosage may prove beneficial.

An alternative explanation for the relatively small therapeutic effect of montelukast on AMS could be that another leukotriene, LTB4, may be primarily responsible for mediating the development of AMS. Richalet et al (31) reported that the increase in plasma LTB4 paralleled the development of AMS. In addition, LTB4 is a potent mediator of capillary permeability (5,7,22). Since the LTB4 receptors are not blocked by montelukast, we cannot discount the possibility that LTB4 mediates development of AMS either alone, or in conjunction with LTC4. Along this line, we did not observe an increase in uLTE4 following 24 hours of high altitude exposure. Previously, we reported an increase in uLTE4 following overnight residence at 4,300 m and a positive correlation between uLTE4 levels and AMS severity (32). We are not certain why we did not observe a similar increase in uLTE4 levels in the present study. It is possible that the previous work was in error. For instance, a recent study (2) also reported no increase in uLTE4 following 20 hours exposure to 4,000 m in a hypobaric chamber. On the other hand, increases in uLTE4 were reported during an expedition to altitudes ranging between 3000 – 5025 m (42). A review of these reports revealed that the studies reporting increases in uLTE4 used only first morning voids for their urine collections. The present study and the other one reporting no increase in uLTE4 collected 12 or 24-hour urine samples respectively, from which the aliquot for uLTE4 assay was taken. Thus, it is possible that the rise in uLTE4 was diluted by the longer duration urine collection period.

A second objective of this study was to evaluate altitude-induced decrements in cognitive performance and whether montelukast administration would ameliorate those decrements. There were no significant effects of montelukast administration on cognitive performance at sea level or high altitude. Regarding the effects of altitude on cognitive performance, two tasks, The Running Memory and Code Substitution Tasks of the SCAT, appeared more sensitive than the other two performance tasks for assessing cognitive performance decrements after ascent to 4300 m. Immediately following ascent to 4300m, impairments in performance of the Running Memory and Code Substitution tasks were observed and these impairments approached about 20% by 4 hours of altitude exposure. Recovery to baseline cognitive performance occurred by 12 hours altitude exposure. Although we did not assess cognitive performance 4 - 12 hr after ascent, we speculate that most recovery occurred shortly after 4 hours. There are three interesting conclusions from these results. First, the results suggest that tasks that are externally paced or require short-term memory recall are most sensitive to the effects of hypoxia. This observation may be useful in designing further studies of altitude-induced cognitive performance impairments and possible countermeasures. Second, the recovery of cognitive performance preceded improvements in arterial oxygenation that require about 7-9 days to fully manifest themselves at 4,300 m (43). This finding suggests that cellular adaptations

not dependent upon increasing systemic oxygen delivery or development of coping strategies facilitated the rapid recovery of cognitive performance at high altitude.

In summary, we administered montelukast, a specific cysteinyl leukotriene receptor blocker, in a randomized, double-blinded, placebo-controlled crossover trial at 4300 m altitude to determine if cysteinyl leukotrienes are involved in the development of AMS. Compared to placebo, montelukast administration decreased AMS prevalence and symptom severity by LL during the first 12 hr exposure, but not after 22 hr altitude exposure. Cognitive performance decrements were not ameliorated by montelukast administration, but recovered to baseline after only 12 hours of altitude exposure. The results suggest a role for the cysteinyl leukotrienes in the development of AMS, but do not rule out the possible involvement of LTB4 in the etiology of AMS.

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